REMARKS

Favorable reconsideration of the instant application in view of the present amendment and the following comments is respectfully requested. Claims 3-5 and 11-15 are currently under examination in the application. By the above amendment, claims 5, 11 and 13-14 have been canceled and claims 3, 4, 12 and 15 have been amended for purposes of clarity and to more clearly define certain aspects of Applicants' claimed invention. Support for the above amendments can be found throughout the specification and claims as originally filed and no new matter has been added. The amendments and remarks herein should not be construed as acquiescence to the Examiner's stated grounds of rejection and are made without prejudice to prosecution of any subject matter modified and/or removed by this amendment in a related divisional, continuation and/or continuation-in-part application.

Applicants acknowledge the objections to the drawings set forth on Form PTO-948 and enclose corrected drawings herewith.

Claims Rejected Under 35 U.S.C. § 112, first paragraph

Claims 3-5 and 11-15 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was allegedly not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. More particularly, the Examiner asserts that the instant specification fails to provide information that would allow the skilled artisan to practice the instant invention without undue experimentation on the basis that Applicants have not demonstrated a nexus between mitochondrial activity, including ATP synthesis, and the treatment of osteoarthritis in a cow.

Applicants respectfully traverse this rejection under 35 U.S.C. § 112, first paragraph, and submit that the instant specification offers more than adequate guidance to allow the skilled artisan to practice the claimed invention without any undue or unreasonable experimentation. Applicants respectfully note in this regard that the claimed invention is drawn to screening methods for identifying compounds that may be suitable in the treatment of arthritic disorders such as rheumatoid arthritis and osteoarthritis, that the instant claims are not directed to

methods for treating osteoarthritis, and that enablement under 35 U.S.C. § 112, first paragraph, should be evaluated accordingly. Moreover, for purposes of clarity, and without acquiescence or prejudice to subsequent prosecution, Applicants have amended claims 3 and 4 such that the claims are drawn to screening methods for identifying agents suitable for altering mitochondrial function in a chondrocyte. Applicants respectfully submit that this claimed invention is unequivocally enabled by the instant specification, as further discussed below, and would be recognized as such by an artisan of ordinary skill.

As disclosed in Applicants' specification as filed, because metabolic energy production in vertebrate articular chondrocytes was previously believed to proceed via anaerobic respiration (*e.g.*, glycolytic ATP synthesis), little or no attention was paid to the relationship between mitochondrial oxidative phosphorylation and arthritic disorders (*e.g.*, rheumatoid arthritis, osteoarthritis). However, according to the instant disclosure, experimental evidence is provided by Applicants that supports a role for chondrocyte mitochondrial function in debilitating diseases such as rheumatoid arthritis and osteoarthritis, enabling new and valuable screening methods, such as those currently claimed, for the identification of compounds that can modulate chondrocyte mitochondrial function.

For instance, in Example 1 of the specification as filed, the mitochondrial ETC complex III inhibitor antimycin A, and the mitochondrial ATP synthase (complex V) inhibitor oligomycin, inhibited respiratory rates and depressed intracellular ATP levels in articular chondrocytes and TC28 cells. These studies demonstrated that antimycin A depleted intracellular ATP levels by 50-80% with less than 10% loss of viability and without altering the constitutively high level of glycolysis, confirming the presence of mitochondrial oxidative phosphorylation in vertebrate articular chondrocytes. The results of additional experiments (*e.g.*, Figs. 2 and 3) showed that after antimycin A addition, the synthesis of both collagen and proteoglycan was depressed by more than about 50% in chondrocytes.

Moreover, in order to confirm that inhibition of mitochondrial function generally impacted collagen and proteoglycan synthesis by chondrocytes, as contrasted with a mechanism by which antimycin A specifically or directly depressed these functions, Example 2 of the specification as filed was carried out with oligomycin, which acts to inhibit the electron transport chain at a different target (complex V) than antimycin A (complex III). Results of this study, in

conjunction with the results set forth in Examples 1, illustrated that impairment of mitochondrial function, which would be expected to occur in some aging chondrocytes, contributed to observed decreases in the synthesis of several extracellular matrix components. Such decreased levels of synthesis of matrix components are hallmarks of arthritic disorders such as osteoarthritis, rheumatoid arthritis and the like.

In Example 3 of the specification as filed, Applicants provided data indicating that healthy mitochondrial function was needed for TGFβ to have its effects on chondrocytes, a point having significance when viewed in light of the fact that TGFβ is thought to play an important role in the repair potential of joint cartilage, especially in arthritis.

In addition, chondrocytes are relatively unique in terms of their ability to elaborate large amounts of inorganic pyrophosphate (PPi). PPi critically regulates both apatite and calcium pyrophosphate dihydrate (CPPD) deposition in osteoarthritis. In Example 4 of the specification as filed, Applicants demonstrated that inhibitors of mitochondrial function attenuated basal PPi elaboration (*e.g.*, Fig. 4). Example 4 further demonstrated that inhibition of mitochondrial function by either antimycin A or oligomycin abrogated the ability of TGFβ to increase extracellular and intracellular PPi. In sum, mitochondrial function, particularly oxidative phosphorylation, appeared to provide ATP that was critical for extracellular matrix synthesis and PPi elaboration in chondrocytes.

Further still, in arthritic disorders such as rheumatoid arthritis and osteoarthritis, matrix vesicles (MVs) are believed to play a role in mediating the pathological deposition of calcium. Accordingly, in Example 5 of the specification as filed, Applicants provided data indicating that mitochondrial functions, including oxidative phosphorylation, modulated the composition and mineralizing activity of matrix vesicles.

Finally, Example 6 of the specification describes screening assays for identifying agents that protect chondrocytes from mitochondrial stressors, such as energy depletion or reactive free radicals. Cells were exposed to one of several agonists added to cultures as stressors for 72 hours, in the absence or presence of candidate chondrocyte protective agents (listed in Table 2). Agents were tested as representative candidate chondrocyte protective agents. Figure 6 shows the protective effect on cell viability (by LDH release) conferred by compounds A, B or C (each at 1 μM) on TC28 chondrocytes exposed to the NO and O_2^- donor

100 μM SIN-1. Similar results (*i.e.*, a moderating effect of the test compounds on NO cytotoxicity) are shown in Figure 7, where TC28 cells were exposed to the NO donor NOC-12 (250 μM) alone or in the presence of 1 μM of compounds A, B or C. Figures 8 and 9 show, respectively, intracellular ATP levels and cell viability of TC28 cells cultured with the NO donor NOC-12 alone or in the presence of 1 μM of compounds D or E. In Fig. 8, intracellular ATP levels are depicted relative to the corresponding control culture that was not exposed to NOC-12, and each NOC-12-treated group displayed significant depression of intracellular ATP regardless of the presence or absence of a candidate protective compound. Fig. 9 shows, however, that despite the decreased intracellular ATP concentrations, significantly higher viability characterized the cells maintained in the presence of either one of the protective compounds. At comparable concentrations (each at 1 μM), compounds J, K and L also demonstrated chondrocyte protective activity in cell viability determinations of cells exposed to SIN-1 or NOC-12. These results illustrate the use of screening assays according to the instant disclosure for identifying candidate compounds capable of protecting chondrocytes from mitochondrial stressors.

In view of the above, it is respectfully submitted that Applicants have provided compelling evidence in the specification as filed that (1) mitochondrial oxidative phosphorylation is indeed active in vertebrate articular chondrocytes, including generation of ATP in articular chondrocytes by aerobic respiration, and that (2) alterations of mitochondrial respiratory activity, and in particular of mitochondrially generated ATP, play a significant role in the pathogenesis of arthritic disorders. Accordingly, it is also respectfully submitted that an artisan of ordinary skill, in view of this disclosure, could understand and practice Applicants' claimed invention drawn to screening assays for identifying compounds capable of altering mitochondrial function in a chondrocyte without undue experimentation and with a reasonable expectation of success.

Reconsideration of the Examiner's rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

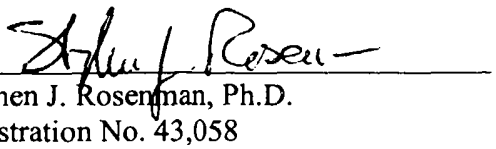
The Commissioner is authorized to charge any additional fees due by way of this Amendment, or credit any overpayment, to our Deposit Account No. 19-1090.

All of the claims remaining in the application are now believed to be in condition for allowance. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

Robert Terkeltaub et al.

SEED Intellectual Property Law Group PLLC



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Enclosures:

Postcard
Check
Petition for Extension of Time
8 Replacement Sheets of Drawings (Figs. 1A-10)

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